APPLICATION OF AMNIOTIC MEMBRANE AND FOETAL MEMBRANES (AMNION & CHORION) IN BURN : A COMPARATIVE STUDY

THESIS

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C E R T I F I C A T E

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INTRODUCTION

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INTRODUCTION

Thermal injury is a serious medical, social and economic problem. Almost every minute of the day, somewhere in the world atleast one human being becomes victim of burns. Burns and its sequelae are often the cause of personal and family tragedies. The world wide development of mechanization and motorization, the growth of heavy industry and of the chemical industry and the wide use of electric energy and ionizing radiation in science and technology contribute to the frequency of burns.

The effect of burns is complex, its treatment expensive, requiring great collective team effort. Many months of hospital treatment are frequently necessary to remove the immediate threat to the patient's life. Reconstructive procedures and therapeutic, vocational and social rehabilitation may last for many years before the patient is able to return to active life.

Burns produce wide raw areas. Coverage of these areas still remains inseparable part of treatment. Since early 19th century idea of autogenous skin grafting to cover the raw area came into existence and is being used. But it has got some limitations (i) if burn area is large, required amount of autogenous donor area is not available (ii) patients are already in shock and not fit for surgery. (iii) it itself produces raw area. Therefore various materials have been suggested by various workers, at different times, either biological or synthetic. Different

biological coverings are homograft skin, heterograft skin, collogen sheets, foetal membranes. Synthetic materials include various films, foams, fabrics, sprays, gels and laminates. One finds it very difficult to chose from such a wide range. An ideal material should have the following properties viz- adherence, water Vapour transport, elasticity, durability, intact bacterial barrier, non antigenic and non toxic, easy to apply and remove and inexpensive or cheap.

Escalating prices of drugs and other materials and phenomenal increase in cost of health care has made it difficult for the commoner to bear the expenses incurred during treatment of burns. As such it would not be inappropriate to develop a suitable efficient and relatively in-expensive treatment for burns that could benefit the poor section of our society. Keeping this in mind, the present study is conducted to evaluate the effect of amniotic and full thickness membranes(amnion & chorion) in burns, and to compare their results.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Ever since man first learnt to make fire, while on the one hand it has served the humanity tremendously, it is considered man's worst enemy for causing burns. The difficulties in the treatment of burns have been lamented by all writers for centuries. The information regarding burns is found in different literature.

Papyrus (1500 BC) had been using boiled cow dung topically. Indian literature shows that Sushutra used mixure of butter with red ochre or the bark of a fig tree. He also recommended debridement of severe burns with loose skin and flesh.

Adams(1939) reports that Hippocrates applied warm mixture over the burn and avoided separation by simple cleanliness. Paulus aeginata (AD 625-690) recommended application of moderate detergent materials which were not definitely heating or cooling.

Rhazes (AD 880-923) had been using white ointment composed of white lead oil of roses and wax. Apart from excision of contracted scars described by Celsus, surgery had no place in the treatment of burns with Greek and Romans. Volesco de Tarenta of Montpellier(1490) described method to avoid syndactyly in the burnt hands.

William Clowes(1544-1604) stands out in history as the first surgeon since the middle ages to use the physical signs of burns 'where the skin was burnt off, and the parts were made raw and painful' to indicate his local treatment.

Hildanus in his book Decombustionibus(1607) insisted that the classification of burns should be a guide for

treatment,classifying burns into three degree by external appearance; redness and blistering; withering and skin without charring; and eschar formation and charring. He warned against cooling burnt skin which would harden the tissues. In very deep burns, he made incisions to let the moisture escape, as otherwise gangrene and infection would supervene. So he was also the first to perform 'escharotomy'.

Wiseman (1676) observed that an organ which is burnt superficially is far more painful than the deep. He too classified burns into three degrees. Heister (1743) suggested a new classification into four degrees, including a time factor as a further diagnostic aid.

Sir James Earle (1799) described several cases of burns which he had treated with ice as an antidote. He suggested that application of cold to a burnt limb alleys pain, and if immediately applied, prevents the formation of blisters and limits the change to an area of erythema. Dzondi(1816) was the first person to carry out controlled experiments on dogs,which were scaled and treated with cold water.

Kentish(1797) justified his mode of treatment with oil of terpentine, which aims to 'diminish the increased action of system'. He had classified burns into two categories.

The Baltimore Medical and philosophical lyceum(1811) described carded cotton dressings, indicating the important principle of dressings a burn 'to give the most perfect protection and comfort'.

Boyer(1814) classified burns into three degrees : Erythema:Blistering leading to superficial ulcers ; and eschar. Dupuytren devided burns into six degrees : First erythema or superficial phlogosis which blanches on pressure. Second cutaneous inflammation , with the loss of

epidermis and the development of vesicles filled with serum. Third the destruction of a portion of the papillary body. Fourth the disorganization of the whole dermis to a subcutaneous cellular tissues. Fifth the formation of eschars of all the carbonization of the whole thickness of the burnt part.

Hebra (1861) treated burn patients in warm water baths. Believing that Dupuytrens last degrees of burns were of academic interest only, he returned to three degree classification (Hebree 1866) Erythema with swelling and pain; blistering with petichial hoemorrhage; eschar and devoid of sensations.

The 19th century presented two basic important principles for the local treatment of burns-skin grafting(Pollock 1871) and open treatment (Copeland Alabama 1887 , Stocker 1894 and Reid 1898). However Bouisson had used open treatment about 400 years back. Lusgarten (1871) suggested and Wilms (1901) carried out excision of burnt tissue for the first time but he never grafted the excised area. Jauschovic (1968) revived the idea of excision of burnt tissue and immediate skin grafting thus making a footnote to the contemporary principle of early tangantial excision and early grafting.

At the termination of 19th century, the combat was to avoid infection and within the 20th century pathophysiology elucidated causes and indicated methods of systemic treatment of burns, counteracting shock. Reiss (1890) and Tommasoli(1897) introduced the systemic treatment of shock by intravenous saline infusions in severely burnt patients. Brown(1896) and Sneve(1905) followed the same suit. But this treatment

became in fourth decade of this century, a routine pratice after the remarkable work of Davidson(1926), Underhill(1930) and Blalock(1931). Previously people had been using alcohalic drinks and opium for correcting shock in these patients.

This was the turning point, and since the mid 20th century, an increased understanding of the metabolic, nutritional, immunological and wound healing process have been recognised improving treatment and comfert of burn patients.

Oppenheimer(1906) advocated the use of picric acid therapy and Davidson (1925) Tannic acid in treatment of Burns. As a consequence, the popularity of the 'exposure' method rapidly declined and only in 1949 was this treatment revived by wallace.

Little pregress had been made from the ancient time in the local treatment of Burns. Man's main concern had been to reduce pain. To achieve this various medicaments from pigeon dung (Aegineta 1535) to enzymatic sloughing agents and antibacterial agents have been used.

Work of Leidbug, Reiss and Artz (1953)indicated septicaemia as primary cause of death in burns and staphylococci as the main organism. With the availability of antibacterial agents against gram positive organism, Pseudomonos emerged as the major organism responsible for sepsis and death. This led other workers to find out antibacterial agents that would penitrate scar. 5% Ag Noz (Moyor), Mefenide (Moncrief), Silver sulphadiazine (Foxe Jr & others), cerium nitrate (Williams W, Monofo, Soun N, Tandan) are the topical agents which minimize bacterial counts over the wound.

Burn Wound Coverings

Thermal injury results in striking anatomic,metabolic and physiologic disturbances which projudice survival of burn

patients. In major degree of burns, the patient is exposed to death from shock or toxaemia due to absorption of poisons from the injured surface or from loss of skin covering or from exhaustion due to the long continued fight for recovery or due to their combined effects.

Autogenous skin grafting is the best covering material, but it has its own limitation in terms of limited supply, unfitness of the already shocked patient for skin grafting. To overcome this problem, various biological and synthetic covering materials, either for short period till the healing of the wound or permanent in place of lost skin, have been suggested by different workers.

Biological Dressings :

Homografts

Pollock(1871), Girdner (1881) and Shede(1881) were the pa pioneers in this field. Ivanova (1890) suggested the advantageous use of foetal skin over the adult because the infantile tissue possessed more "energetic vitality" .

Dago(1952) introduced the use of Postmortem allografts as temporary biological dressings. The use of fresh Postmortem allografts have added measurably to the successful treatment of burns. As temporary biological coverings, they decrease fluid and protein loss,diminish infection and prepare granulating surface for the application of autogenous grafts.

Brown(1952) used allografts as emergency dressings for burn. He stated that the skin may be removed even days after death if the cadaver has been placed in cold storage.

Eade (1958) and Morries(1960) observed that the homografts have organizational and debride-mental effects on healing wound.

Healed epidermis shows alteration in the architecture and the dermis contains aedematous connective tissue in 2nd degree burns where homografts are not used. When the homografts are used, the healed epidermis shows normal architecture with recognizable basal layer and normal collagen bundles in the dermis (Miller 1967).

James O'Neill Jr(1967) used temporary homografts coverage over open wounds including 2nd and 3rd degree burns. Such coverage was of distinct benefit following eschar separation in burn injury. Sharma et al(1978) reported the same results.

Allografts skin, besides being satisfactory, biological dressings have their own limitations. Cadavers suitable for skin donations are limited in number. Bexter(1970) has estimated 6 physician hours and hospital cost of $225 per patient needed to use cadaver homografts.

Xenografts

The use of Heterologous tissue as a temporary dressings for full thickness skin defects was largely a result of the difficulty of obtaining adequate amount of homografts. Brown, Burleson and Lewis have shown that the adherence of allografts and xenografts is similar. Heterograft provides a readily available, easily stored and sterilized dressings in contrast to homografts. The only xenograft in common use is pig skin. Variable results have been reported with porcine xenograft coverage of donar sites and partial thickness burns ranging from early re-epithelization to conversion of full thickness skin loss. Salisbury(1973) has reported that incorporation of xenograft tissue on healing donar sites occurs in 35 percent of cases. There appears to be no significant difference in the effectiveness of fresh

compared with fresh frozen or frozen irradiated procine skin.

The most striking advantage with the procine xenograft is that of immediate and lasting pain relief. Xenograft has most of the properties of the ideal skin substitute. A viable xenograft is [illegible] antigenic but the dead is not. The major problem is the propensity to digestion by wound collagenase and subsequent infection.

Collagen Sheets

Collagen, fibrous protein is present in many animal tissue like skin, muscle and bone. Its structure and immunologic chemistry are well characterized and antigenicity can be altered. It also possesses a haemostatic effect; when implanted in pure from over a living animal tissue, no antigenic reaction is seen. Air borne infection is minimised and fluid loss is prevented. Thus it isideal raw material to be used in burns. It is derived from serous and sub serous layers of freshly slaughtered cattle intestine and are commercially available in 10 cm.X 15 cm size packed in cylindrical glass tubes containing ethylene oxide which acts as sterilizing agent.

Sinha (1972), Shankar (1975) and Gupta et al (1976) used collagen sheets as primary cover material in the managemnet of burns. Elhans et al (1978) used sheets as biological dressings in 22 patients and reported its role in prevention of infection and in increasing the rate of healing. Jain et al (1976) reported the similar findings.

Synthetic Materials

The problem associated with biological materials provided an impetus to search for synthetic material with ideal properties for a skin Prosthesis. Earlies research work was a sulfonamide film (Pickrell, 1942). Many of these materials

adhere by intrapment of coagulum in the interstices of the material. Silicon polymer membrane is the best material available because it is elastic, durable and the water vapour transfer characteristics can be controlled by varying the thickness. Kornberg et al (1972) have used thin silicon membrane bonded to cotton gauze for temporary skin substitution but it lacks elasticity and creats non uniform pattern of adherence. Other materials are modified polyvanyl chloride or similar plastics which provides more elasticity and water vapour transfer characteristics. (James et al, 1975, Lamkay et al, 1977, Towsend, 1977). The material is deep but the greatest disadvantage is lack of adherence to wound itself. These materials seem to have great promise as a temporary skin substitute for short time applications.

Amniotic Membrane

The quest for a cheap, painless and easily available biological dressing having most of the properties of the ideal skin substitute led people to use amniotic membrane.

Amniotic membrane is the inner of the two foetal membranes having two surface. Its outer surface is separated from the deciduous of the maternal uterus by chorion. The inner surface is in contact with the content of the amniotic sac i.e. the fluid and the foetal body.

A section of amnion under a light microscope shows 5 different layers :

1. Epithelium : This is a single layer of non ciliated cuboidal cells having a role in the exchange of fluids and electrolytes between amniotic sac and mother.
2. Basement membrane : This is a narrow band of reticular tissue at the base of epithelial layer adherent to it.

3. Compact layer : It is a dense acellular layer deep to basement membrane. It is firmly adherent to the basement membrane and cannot be separated from it normally.

4. Fibroblast layer : It is composed of fibroblast net work present in mesh of reticulum. Fibroblast and hofbour cells (Macrophages) are normally present in this layer. It forms a considerable part of the thickness of amnion.

5. Spongy layer : It is composed of extra embryonic coelomic reticulum. It is capable of great distension. It contains mucous in its structure which enables the layer to alter its shape.

The normal thickness of the membrane is 1/50 to 1/2 m.m. which may increase to as much as 2-5 cm. due to change in the amount of the mucin and fluid within the spongy layer and the capability of loose connective tissue to great variation.

The Chorion : The chorion consists of four layers, these are from within outwards:

1) Cellular layer : thin layer consisting of an interlacing fobroblast net work.

2) Reticular layer : It forms the majority of thickness of the fibrous chorion and consists of a relicular net work, the fibres of which tend to be parrallel. Nodes present on the fibres at those places where branching occurs.

3) Pseudo basement membrane : It is a layer of dense argyrophil connective tissue that is firmly adherent to the reticular layer above.

4) Trophoblast: It forms 2 to 10 layers of trophoblast cells in contact, on their deeper aspect, with material dicidua. This layer contains obliterated chorionic villi.

Blood Supply

Amnion does not have any blood supply at term as well as not at any stage of Pregnancy.

Nerve Supply: Nerve supply have not been described in the amnion but the findings have not been confirmed.

Lymphatic Vessels: Possibilities that amnion contains lymphatic vessels have been suggested by some workers.

Embryonic development :

In the human embryo, development of amnion begins during the transformation of morula to blastocyst stage at the time of implantation, about 7-8 days after fertilization. There is separation from inner all mass of the germ disc at the periphery of the ectodermal layer of polyhedral cells. " Amniogenic cells", to form a slitlike cavity, with appearance of primary extra embryonic mesoderm. The amniotic mesenchyme is derived epithelium becomes separated from the primitive trophoblast. Amniotic mesenchyme is derived from the primary extra embryonic mesoderm of the trophoblst.

Finally the foetal membrane consist of an inner amniotic membrane consisting of a single layer of ectodermally derived amnion cells. Collagen rich mesenchyme of 6-8 cells in the thickness. The chorion consisting of compressed trophoblastic tissue of the chorion leave and mesenchymal tissue.

Immunology

Various studies have been carried out to observe the facts concerning this important aspect of the subject.

Amnion when implanted to its own newborn infant"takes" as a permanent graft.Neovascularization has not been seen.Nourishment of the graft appears to be by simple diffusion.

When implanted sub cutaneously as allograft,results were similar to autograft for first 14-17 days. Later on these grafts were transformed into hylinised substances . Similar results

have been observed when amniotic membrane was used as biological dressing. When the mesenchymal surface was placed towards the host "superior take up" or "fixation" was seen, while on placing amnion towards host, little fixation was noticed at the end of 72 hours. No neovascularization was observed in any case.

The allograft amnion membrane appeared viable histologically after 21 days. When it was placed in pelvic cavity after pelvic exenteration, granulation tissue and fibroblast tissue activity was markedly inhibited as compared to control cases.

When allograft amnion was implanted intra peritoneally in the experimental animal in whom the caecum was damaged and contaminated, prevention of adhesions and gradual disintegration of membrane without any host response occured.

These experiments suggest that antigenicity of amnion is low and no voilent host reaction noted yet.

When the chorion was placed over host tissue as autograft and allograft neovascularization and migration of host cells was observed. It provokes strong cellular and less antibody response. The tissue had an accelerated rejection phenomenon in 72 hrs, being rejected by 11th day. This rejection phenomenon can be delayed by high doses of progesterone.

Clinical and experimental application

In 1910, Davis, a senior medical student reported attempts at grafting pieces of the lining of the amniotic sac onto granulating wounds.

Sabella(1912) for the first time treated a brunt patient, applying amniotic side of the membrane towards the wound because of its ectodemual origin and reported results as: Absence of infection, reduction in pain and rapid re epithelization. While Burger(1937) used Amnion in construction of vagina; De Roth(1940) reported its sucessful use in conjunctival repair.

Chao et al(1940) and Kohnstan (1941) suggested the use of "Amnio plastin", a preparation made by the immersion of amnion in alcohal for fixing, followed by drying in sheets and boiling in water for 20 minutes for sterilization. This was washed in normal saline before use. This fixed, dead amnion preparation was employed as a covering to prevent adhesions during operations on the brain. Later Pinkerton(1942) and others used it for mobilizing adherent tendon fixed by adhesions.

The credit for using living amnion as a homograft in burns goes to Kubani, a Hungarian. He also used sterile amnion to cover raw surface of the peritonium after separating adhesions. His contemporary, Henson(1950) used amniotic membrane in the management of chronic skin ulcerations with smooth side of amnion facing wound. He observed that granulation tissue never raised above margins which occured when plaster of paris was used to cover the wound.

Douglus(1952) used Heterografts(human skin to chorio-allentoic membrane of chick and human amnion to chick amnion) and reported successful results. He further applied heterografts in experimental dogs using amnion and chorion over the wounds. Healing was quick and infection was less and he noticed that dressings separated readily from the surface, grafted with amnion, leaving a shiny, dry and pinkish surface. The chorion grafts were more opaque and more salmon pink coloured.

Douglus(1951)also did the first successful homografting in an extensively burnt patient.

Douglus and other(1954) tranplanting homologous and heterologous chorionic membrane to the mouse concluded that homologous and heterologous grafts of foetal membrane are

tolerated as covering for open wounds as well as homologous grafts of skin and for two or three times the duration of the homografts of the skin. The transplants of foetal membrane served as viable transplants, capable of cellular division and epithelization. Their experiments indicated that chorionic grafts may be more useful as temporary coverage of wounds than are homoplastic skin grafts.

Jullian (1956) using the amnion membrane over old infected flame burns successfully, suggested the amniotic membrane as dressing material for emergency measurement of trauma.

Henson(1956) treated patients with intermittent claudication, grafting amniotic membrane and found favourable results. Similar results were reported by Rowling(1958) Unger-Hamiltan(1958) and Hansen(1960).

Pigeons(1960) contention in using amniotic membrane was that it is similar to skin as it arises from ectoderm of the foetus.

Massee et al (1962) reported favourable results with amniotic membrane grafts in experimental dogs where he carried out pelvic exenteration. They showed that there were few adhesions and dense scar formation.

Dino(1965) in his study using various layers of foetal membrane on burnt patient, observed that following application of membrane, there was immediate relief in pain. However, he did not report any allergic reaction, crust formation was there over grafted areas which remained dry and uninfected untill their pealing off(9th to 20th day) following grafting. He used amnion, amnion with chorion and chorion alone and did not find any difference after using these

layers.

Dino's next study(1966) was to find out the best preservative. He preserved the amniotic membrane in different sterile solutions at 4^{0}c Temp. viz.1 Normal saline 2.Benzal konium solution(1:1000 dilution) 3. sodium hypochloride (1:40 dilution) in normal saline solution 4. saline solution(500 cc) with 1 million units of crystalline penicillin and 1 gr. of streptomycin sulphate or Kanamycine. Amniotic membranes were preserved from fresh stage to one month and were used in treatment. Bacteriological examinations of membranes were done on 1st, 3rd, 7th and 30th day. He labelled solution of sodium hypochloride 2. Solution of crystalline penicillin with streptomycin sulphate or Kanamycin sulphate as best.

Galask et at(1970) observed the presence of several factors in the human amniotic membrane which are known to be antibactenal. They have clearly shown inhibition of number of bacterial genera by amniotic fluid, even by amniotic fluid supplemented by casein hydrolysate.

Trelford et al(1970) confirmed the observation of Douglus that the mesenchymal side towards the host provided more consistent 'take' while using amnion as auto and allografts in experimental sheep.

Robson et al(1973) studied the effect of human amniotic membranes on the bacterial population of infected rat burns. They concluded that compared to human skin, the amniotic membrane was more effective at decreasing the bacterial counts in the burn wounds. They sought specific antibacterial substance using invitro techniques with amniotic membrane homografts but no such substance was found, and proposed that the invitro antibacterial effect seen is due to achievement of a biologically closed wounds by the membrane, thus allowing

the host's own defence mechanism to deal with the bacterial population as did other biological dressings.

Robson et al (1973) treated 50 patients having open wounds with full thickness amniotic membrane. Over full thickness defects, the amniotic membranes were placed on the wound with the chorion against the granulating surface , changing them at every 48 hours. Before applying membrane and while changing the membrane, specimens were taken for bacterial analysis. In partial thickness wounds, membranes were applied with chorion facing the wound and in some, amnion facing the wound. They observed that amniotic membrane adhered to all wounds regardless of their depth. In all of the full thickness wound, the bacterial count decreased and the decrease was equal to allograft skin and superior to xenograft skin.

Colocho & others (1974) observed the effect of human amniotic membrane in clinical and experimental studies. Amniotic membranes were used to cover the split thickness donar sites and partial thickness burn wounds. In rats, open wounds were covered with amnion and in some, it was placed in sub-cutaneous pockets. None of the biopsy specimens showed vascularization of the amnion by the host. The membrane became dissicated forming a dry eschar over the surface of burn that gradually became detached as reepithelization proceeded beneath the membrane.

Bapat and Kothari(1974) successfully used living amniotic membrane grafts for the restoration of the floor of the mouth in the patients of advanced cancer of the tongue, following radical total glossectomy. They observed that the healing was rapid with induction of metaplasia in a

fore-night. Grafted area showed hardly any scarring. The floor remained flexible and pliable.

Trelford- Souder and other (1977) used the amniotic membrane to cover the raw area after pelvic exenteration and innumerated advantages as : Readily availability of low antigenic tissue, lack of intestinal complications, reduced fluid and protin loss, technically easy method, reduced hospitalization and reduced number of intro-abdominal adhesions.

Bose B(1979) used membrane as biological dressing's over burns, pointing out that amnion adheres more firmly than any other biological dressing. Agarwal V K (1982) reported similar results.

MATERIAL AND METHOD

MATERIAL AND METHOD

The present study has been conducted at M.L.B. Medical College Hospital, Jhansi from May 1982 to April 1983 to compare the effects of amniotic membrane and Full thickness foetal membrane (Amnion+Chorion) over thermal burns as bio-dressings.

Collection of membranes

The amniotic membrane and Foetal membranes were collected from the labour room and obstetric operation theatre of M.L.B. Medical College Hospital, Jhansi at the time of labour and caesarian section. The mothers having intact membranes and without any history suggestive of genital tract infection were selected. Parity and blood groups of mothers were not considered.

Separation of Membranes from placenta

The placenta with intact membrane was taken directly in a clean tray and was washed thoroughly in running tap water to remove blood and mucoid material then it was transferred to another clean tray filled with water. For full thickness membrane intact chorion and amnion was cut around placental margins and for amniotic membrane, it was separated from chorion starting from periphery upto the base of umbilical cord and was cut around . The separated membranes were spread over a flat surface in a sterile container filled with sterile normal saline and any remaining clots were removed gently from its surface with the help of sterile gauze pieces. These membranes

were again rinsed in sterile normal saline for four-five times.

Preservation of membranes

Membranes thus obtained were either applied immediately to burn area or kept separately in sterile normal saline treated with 10 lac units of Benzyl penicilline and 1 gm of Streptomycin sulphate and preserved at 4^{o}c till the time of application. The preserved membranes were continuously watched for bad odour or change in colour and texture. Membranes preserved for more than one month period were not used.

Selection of cases

All the cases having burn less than 50% of body surface, either deep or superficial, who came to emergency department or out patient department of this hospital within 72 hours of the thermal injury were included in this study, irrespective of their age, sex socio economic status, contamination of wound and mode of injury.

Method of Study

The selected cases were subjected to detailed history and physical examination which were recorded on following lines :-

(i) History

Introduction : Name, Age, Sex, Occupation, rural/urban, address, date of admission, date of discharge and time of healing .

- Date and time of burn (duration)
- Place of accident and nature of work at the time of accident.
- Cause of burn

- Prior treatment (if any)
- Symptoms

(ii) Physical examination

General Examination: The case was examined for general condition, pulse, blood pressure, temperature, respiration and hydration.

Local Examination :

(A) Percentage of Burn : It was calculated by "Wallace's rule of Nine" in the adult and by "Lund and Browder Chart" in children.

(B) Depth of Burn : Superficial/deep.

Estimation of depth of Burn:

The hypodermic needle was used to test the pain sensation. The area with increased sensibility was considered to be superficial or partial thickness burn. The area with markedly reduced or absent pain sensibility was considered to be deep or full thickness burn. This was also confirmed by pulling out a hair from burn surface. In the third degree or deep burn hair pulls out easily and without pain. This test is of value in borderline cases of second degree burn. In addition, help of the following criteria was also sought :

Classification of depth	Appearance of Burn area	Pain sensation
I degree	Erythematous	Painful and hyperaesthetic.
II degree		
(A)	Blisters with reddened base and moisture	Painful and hyperaesthetic
(B)	Blisters with Blanched base and moisture	Painful, hyperaesthetic or anaesthetic at places
III degree	Leathery pale or pearly white or charred dry.	Painful and anaesthetic

The I and II (A) were considered as superficial and II (B) and III were considered as deep burn.

(C) Contamination of wound

Apparently clean : No contamination of foreign body, clean intact blisters.

Mild contamination : Slight contamination, ruptured blisters, open wounds.

Gross contamination : Heavy contamination with dirty cloth, foreign body, dust and/or cowdung, mud etc.

(D) Area involved : Diagramatic representation of area involved was made.

Resuscitation and general treatment

Prior to application of membranes, patients were resuscitated and general treatment was given to every patient(i.e. $^{1}/_{v}$ infusians, blood and plasma infusian, analgesic, antibiotics and tetanus prophylaxis).

Local management of wound

Patients were devided into following groups :

Group 'A' : Amniotic membrane was applied over full burn area.

Group 'B' : Full thickness foetal membrane was applied over full burn area.

Group 'C' : Amniotic membrane was applied over one part (C_1) and full thickness membrane on ether part (C_2).

Procedure :

The burnt areas to be grafted were prepared by throughly debriding the dead skin and cleaning them with 5% savlon solution and sterile saline solution. Spirit was applied over adjacent normal skin. After this burnt areas were again assessed for degree and percentage of burn.

Fresh or preserved membrane (amniotic membrane or full thickness foetal membrane) was stretched out and was applied on the burnt surface. The application was done in such a way that the membrane extended beyond the borders of the burn, overlapping the normal skin. This was done to help keep the membranes in place since it adheres easily to dry skin. The amniotic membrane was applied with smooth surface facing the wound while full thickness membrane was applied with chorion facing the wound.

In movable areas like the exhimities and joints, the graft was held in place by covering it first with sterile gauze then bandaging with sterile rolled gauze. In relatively immovable parts like the chest and abdomen, the membrane was left alone as applied without any additional dressing.

No anaesthetics were used on the burn areas before membrane application.

Assessment of the case

The assessment of the results was done daily following the application of the membranes.

The patients were asked about :

1. Pain and discomfort prior and after application of membranes.
2. Fever
3. Any evidence of allergy as itching, rashes, nausea, vomiting.

Physical Examination

General Examination: Patients were examined for general condition, hydration, pulse, blood pressure and signs of toxaemia.

Local Examination : Observation for the following was done :

(1) Presence of discharge and /or soakage.

(2) Appearance of membranes as regard to surface, margin, thickness, lustuure, colour, dryness and adherence.

(3) Collection of Pus under dressing: if the Pus was localized in small area underneath membranes, a slit was given in it and pus was squeezed out. A Pus swab was taken for culture and sensitivity. If the pus was present underneath th the whole of the membrane then membrane was removed, Pus swab was taken for culture and sensitivity and wound throughly cleaned. The 2nd application of membrane was done after control of infection.

1) Result of healing

Investigations :

1. Routine - Complete blood haemogram
   - Urine- gross and microscopic examination.
2. Culture and sensitivity test for Pus if present.

Pus swab was taken for culture and sensitivity and antibiotics were given according to reports.

PROFORMA

Name

Age/Sex

Occupation

Rural/Urban

Address

Date and time of admission.

Date and time of discharge.

Group

Total time of healing

HISTORY

(i) Date and time of burn

(ii) Place of work and nature of work at the time of burn

(iii) Cause of burn

(iv) Prior treatment (if any)

SYMPTOMS

(i) Pain

(ii) Burning

(iii) Blisters

(iv) Fever

(v) Oliguria

(vi) Discharge from wound surface

(vii) Difficulty in swallowing or in inspiration

(viii) Any other

PHYSICAL EXAMINATION

(a) General examination at the time of admission

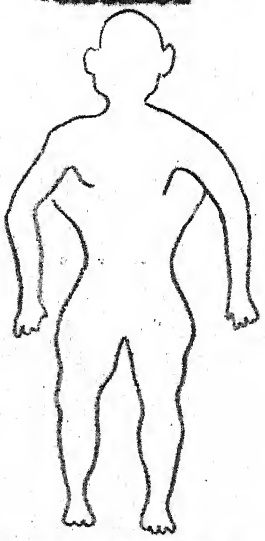
-G.C. -Pulse -B.P.

-Temperature - Hydration

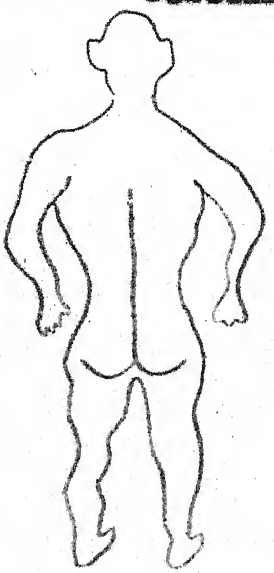
(b) Local examination

- Percentage of burn
- Depth of burn/Degree of burn
- Contamination
- Appearance of raw surface area
- Area involved (Diagramatic)

Anterior



Posterior



Lateral

Progressive report -

Days	1	2	3	4	5	7	9	12	14	18	21	25	30	35
G.C.														
Pulse														
B.P.														
Hydration														
Temperature														
Difference between input & output														
Albumin in Urine														

	Wound dressed with amniotic membrane													Wound dressed with full thickness membrane										
Days	1	2	3	4	5	7	9	12	15	18	22	25	30	1	2	3	4	5	7	9	12	15	18	22
Pain																								
Soakage																								
Mobility																								
Biodressing changes -																								
(a) Surface																								
(b) Margins																								
(c) Thickness																								
(d) Lusture																								
(e) Colour																								
(f) Dryness																								
(g) Adherence																								
Healed on																								
Time of healing																								

Investigations

Blood - TLC
DLC
Hb%
ESR

Urine - Albumin
Sugar
M/E

Pus - Culture & Sensitivity

Treatment

(i) I/V fluids
(ii) Blood
(iii) Sedative
(iv) Analgesics
(v) Systemic antibiotics
(vi) Local application

Photograph-1: Showing seperation of amnion with chorion from the placenta .

Photograph-2: Showing seperation of amnion from chorion.

Photograph-3: Showing seperation of amnion upto the base of umbilical cord.

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OBSERVATIONS

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OBSERVATIONS

The present study consists of 31 patients of Burn admitted in surgical and emergency wards and also as O.P.D. cases in surgery at M.L.B. Medical College, Jhansi from may 1982 to April 1983. These patients belonged to different social strata and were of either sex. These patients had age group ranging from 3 months to 50 years and had burns involving less than 50 per cent of total body surface area. only those cases were included who reached hospital within 72 hours of having sustained burn injuries.

Of the total 31 patients, 30 patients were below 30 years of age. 9 cases (2 males and 7 females) i.e. 29.032% were below 10 years of age, 10 cases (5 males and 5 females) i.e. 32.258% between 11-20 years of age and 11 cases (5 males and 6 females) i.e. 35.484% were between 21-30 years of age. The only patient above 30 years of age was a male of 50 years. Total male patients were 13(41.936%) and female patients 18 (58.064 %).

Table No. 1

Showing age incidence

S.N.	Age groups	Number of cases	% age
1.	0 - 10	9	29.032
2.	11 - 20	10	32.258
3.	21 - 30	11	35.484
4.	31 - 40	0	-
5.	41 - 50	1	3.226
	Total	31	100

Table No.2

Showing Sex incidence in difference age groups

S.N.	Age groups (Years)	Male		Female	
		Number	%age	Number	% age
1.	0 - 10	2	6.452	7	22.580
2.	11 - 20	5	16.129	5	16.129
3.	21 - 30	5	16.129	6	19.355
4.	31 - 40	-	-	-	-
5.	41 - 50	1	3.226	-	-
	Total	13	41.936	18	58.064

Maximum burn injuries 26 (10 male and 16 female) i.e. 83.871% occured at home when the patient was engaged in his daily home tasks. 5 patients (16.129%) sustained burns while working out door, of these 3 were male and 2 female.

Table No. 3

Showing location of Burn accident in different Sex

S.N.	location	Male		Female	
		Number	% age	Number	% age
1.	Indoor	10	32.258	16	51.613
2.	Outdoor	3	9.677	2	6.452
	Total	13	41.936	18	58.064

14 (45.161%) out of 31 patients hailed from rural area and 17(54.839%) from urban.

Table No. 4

Showing rural/urban incidence

S.N.	Rural/ Urban	Male	Female	Total	% age
1.	Rural	7	7	14	45.161
2.	Urban	6	11	17	54.839
	Total			31	100

It was observed that 21 patients (15 females and 6 males) i.e. 67.74% sustained burn injuries from fire while

cooking food. 3 patients i.e. 9.667%(2 male and 1 female)suffered burn injuries from fire. 5 patients i.e. 16.129% (3 male and 2 female) had burns due to scalding and one male patient suffered injuries while working on electric installation. 26 cases were superficial and 5 were deep burns.

Of the 31 patients, 7 were housewives, 3 farmers, 12 students, 1 electrician and 1 patient was doctor who suffered injuries from fire-crackers.

Table No.5

Showing cause of burn according to sex

S.N.	Cause of Burn	Male		Female		Total	
		Number	% age	Number	%age	Number	%age
1.	Fire during work or cooking	6		15		21	67.742
2.	Lamp	2		1		3	9.677
3.	Scalding	3		2		5	16.129
4.	Electric Current	1		-		1	3.226
5.	Miscellaneous	1		-		1	2.226
	Total	13		18		31	100

Table No.6

Showing different occupation among the burn patients

S.N.	Occupation	Number of cases	% age
1.	Housewives	7	22.581
2.	Farmer	3	9.677
3.	Labourer	1	3.226
4.	Student	12	38.710
5.	Electrician	1	3.226
6.	Professional	1	3.226
7.	Miscellaneous	6	19.355

16 patients i.e. 51.613 out of 31 reached hospital within 12 hours of injury. 8 patients (25.806%) reached within 13-24 hours. 6 patients (19.355%) attended hospital within 24-48

hours and one patient(3.226%)reached hospital after 70 hours of injury. Off all these cases,14(45.161%)were apparently clean wound, 13(41.935%)mild contaminated and 4(12.903%)were grossly contaminated wound.

Table No.7

Showing time interval between burn accident and hospital arrival

S.N.	Duration in hours	Number of cases	% age
1.	0 - 12	16	51.613
2.	13 - 24	8	25.806
3.	25 - 36	4	12.903
4.	37 - 48	2	6.452
5.	49 - 60	0	-
6.	61 - 72	1	3.226
	Total	31	100

Table No. 8

Showing the grade of contamination of wound at the time of admission

S.N.	Grade of contamination	No. of cases	% age
1.	Apparently clean	14	45.161
2.	Mild contamination	13	41.935
3.	Gross contamination	4	12.903

In 11 cases (35.494%) membranes were applied within 12 hours of injury, in 9 within 12-24 hours, and in another 9 between 24-48 hours. Only in one case membrane was applied within 49-60 hours and in one case after 70 hours of injury.

Table No.9

Showing time interval between burn accident and application of membrane

S.N.	Time interval in hours	No.of cases	% age
1.	0 - 12	11	35.484
2.	13 - 24	9	29.032
3.	25 - 36	6	19.355
4.	37 - 48	3	9.677
5.	49 - 60	1	3.226
6.	61 - 72	1	3.226
	Total	31	100

Out of 31 cases to whom membranes were applied, 15 cases (48.387) were having less than 10% burns, 8 cases 11-20 %, 3 cases 21-30% and 5 cases were having 31-40% of burns. These were devided into three groups. In 18 cases, only amniotic membrane was applied, in 8 cases, Amnion with chorion was applied and in 5 cases both membranes were applied to different wound areas.

Table No. 10

Showing Percentage of burn

S.N.	Percentage of burn	Number of cases	% age
1.	0 - 10	15	48.387
2.	11 - 20	8	25.806
3.	21 - 30	3	9.677
4.	31 - 40	5	16.129
5.	41 - 50	-	-
	Total	31	100

Table No. 11

Showing the number of cases in different group according to local management of wound

S.N.	Group	No. of cases	% age
1.	A	18	58.065
2.	B	8	25.806
3.	C(C_1+ C_2)	5	16.129
	Total	31	100

Thus total 31 cases with 36 wound areas were considered. In 23 wound surface, only amniotic membrane was applied and 13 wound surface, Amnion with chorion was applied.

Table No. 12

Showing the percentage of Burn surface over body treated by different group.

S. No.	Percentage of burn	Total Amniotic Membrane				Total full thickness membrane			
		Treated Wound		Total	%age	Treated Wound		Total	%age
		A	C_1			B	C_2		
1.	0 - 10	9	2	11	47.826	6	1	7	53.846
2.	11 - 20	6	2	8	34.783	1	4	5	38.462
3.	21 - 30	1	1	2	8.696	1	-	1	7.692
4.	31 - 40	2	-	2	8.696	-	-	-	-
5.	41 - 50	-	-	-	-	-	-	-	-
	Total			23	100			13	100

Total area 36

In the clinical observations, following main criteria were considered viz., relief of pain and discomfert, development of fever and allergic reaction, control of oozing and gross infection. The duration of healing and condition of wound after separation of membrane were also compared in two different types of membrane application.

Out of 23 amniotic membrane treated wounds, immediate relief of pain and discomfert was recorded in 22 cases(95.652%) and in only one case, analgesics and sedatives were required. Similarly of the 13 full thickness membrane treated wounds, only one patient(7.692%) required analgesics and sedative for persistent pain and discomfert. No allergic reaction was seen in any case. Fever was noticed in 7 cases, all development infection.

Table No.13

Showing the immediate effect of different dressings

Type of dressing	Group	Total wounds	Pain and discomfort						Allergic reaction
			Relieved			Persisted			
			No.	Total	% age	No.	Total	% age	
Amniotic Membrane	A	18	17	22	95.652	1	1	4.348	-
	C_1	5	5			0			-
Full thickness Membrane	B	8	8	12	92.307	0	1	7.692	-
	C_2	5	4			1			-

In 26 cases, oozing stopped within 24 hours of application of membrane. In one case, oozing stopped within 36 hours and in 4 cases, oozing stopped in 36-48 hours. All these cases were deep Burns.

It was observed that in all wounds treated by amniotic membrane, it became dry and adherent to wound surface within few hours of application (6 hours- 12 hours) whereas full thickness membrane took longer time (12-24 hours) to become dry and adherent to wound surface.

In about 24-48 hours, both types of membrane covering the adjacent normal healthy skin started curling up but remained adherent to wound surface. In about 2 days, amniotic membrane became opaque and as membrane dried, its colour changed from yellowish to light brown. Full thickness membrane was white opaque when applied and as it started drying, it became more transparent in 24-48 hours and then its colour changed dark yellowish brown.

23 of the membranes(17 Amnion and 6 Amnion with chorion) were used within 6 hours following procurement. 5 Amnion with chorion and 3 Amnion were used after being preserved for 1 day, one Amnion and 2 Amnion and chorion after 3 days, one Amnion

after 6 days and one Amnion after 15 days and no differences were noticed with regard to using the membranes in various duration of preservation.

7 wounds (6 to which amniotic membrane was applied and one to which full thickness membrane was applied) developed soakage. Culture showed that soakage was due to pus formation in these cases.

Table No.14

Showing the incidence of soakage and Pus formation after 2 different type of dressings.

Type of dressing	Total wound	Soakage		Pus formation	
		No.	% age	No.	% age
Amniotic Membrane treated wound	23	6	26.087	6	26.087
Full thickness membrane treated wound	13	1	7.692	1	7.692

Two amniotic membrane treated wounds developed staphylococcal infection, two developed pseudomonas infection, one wound developed staphylococcus and Klebsiella and one wound developed staphylococcus and E coli infection. One full thickness membrane treated wound developed Pseudomonas infection.

Table No. 15

Showing Bacteriology of wound surface treated by two different types of dressings.

Type of Bacteria	Amniotic membrane treated wound	Full thickness membrane treated wound
1. Staphulococcus	4	-
2. Pseudomonas	2	1
3. Klebsiella	1	-
4. E coli	1	-

Of the 23 wounds treated with amniotic membrane, 3 wounds (13.043%) which involved 0-10% of body surface area, healed in 6-10 days, 10 wounds (43.478%) healed within 11-15 days time of which 4 had 0-10% of Burns, 3 had 11-20% of burns, 2 had 21-30% of burns and 1 had 31-40% of burns. 4 wounds healed within 16-20 days time, 2 having 0-10% burns, 1 involving 11-20% burns and 1 involving 31-40% Burns. One case involving 18% of Burns in leg expired on 20th day due to septecaemia. 3 wounds (113.043%) healed in 21-25 days, 2 involving 0-10% Burns and 1 involving 11-20% Burns. 1 wound (4.348%) involving 11-20% Burn surface healed in 26-30 days and one wound(4.384%) involving 11-20% Burn surface healed in 36-40 days.

Of all 13 wounds treated with full thickness membrane, 7 wounds i.e. 53.846% (6 involving 0-10% of body surface and one wound involving 21-30% of body surface), healed in 6-10 days, one wound (7.692%) involving 11-20% of body surface in 11-15 days. Two wounds (15.385%) having 11-20% of Burns healed in 16-20 days. Two wounds (15.385%), one having 0-10% Burns and one having 11-20% Burns healed in 21-25 days time. One wound (7.692%) involving 11-20% of body surface took 36-40 days to heal.

Out of 36 wounds in total rejection of membrane occured in 5 Amniotic membrane treated wounds and 1 full thickness membrane treated wound. Pus collection underneath the membrane appeared to be the main cause of rejection. In these cases membrane were reapplied after control of infection. In 2 of 6 cases where membranes were reapplied, rejection again occured probably due to failure of control of infection. These cases were later treated by antibiotic gauze piece and

Showing the rate of healing in burns treated by the different types of dressings in different of % age of Burns.

Total days of healing	Amniotic membrane treated group (23)							Full thickness membrane treated group(13)						
	Percentage of Burn areas					Total		Percentage of Burn area						
	0-10	11-20	21-30	31-40	41-50	No.	%age	0-10	11-20	21-30	31-40	40-50	No.	%age
6-10	3	-	-	-	-	3	13.043	6	-	1	-	-	7	53.846
11-15	4	3	2	1	-	10	43.478	-	1	-	-	-	1	7.692
16-20	2	1	-	1	-	4	17.391	-	2	-	-	-	2	15.385
21-25	2	1	-	-	-	3	13.043	1	1	-	-	-	2	15.385
26-30	-	1	-	-	-	1	4.348	-	-	-	-	-	-	-
31-35	-	-	-	-	-	-	-	-	-	-	-	-	-	-
36-40	-	1	-	-	-	1	4.348	-	1	-	-	-	1	7.692
Total						23							13	

One patient with 11-20% of Burn expired on 20th day.

Table No.17

Showing the result after two different types of membrane application

Type of dressing	%age of Burns	Total wound surface	Rejection	Reapplication			Contracture	Keloid
				Accepted	Again rejected	Mortality		
Amniotic Membrane treated wounds	0-10	11	3	3	-	-	-	1
	11-20	8	1	-	1	1	1	-
	21-30	2	-	-	-	-	-	-
	31-40	2	1	-	1	-	1	-
	41-50	-	-	-	-	-	-	-
		23						
Full Thickness membrane treated wounds	0-10	7	-	-	-	-	-	1
	11-20	5	1	1	-	-	-	-
	21-30	1	-	-	-	-	-	-
	31-40	-	-	-	-	-	-	-
	41-50	-	-	-	-	-	-	-
		13						

systemic antibiotics according to culture and sensitivity of Pus. All these cases were either grossly or midly contaminated at the time of admission in the hospital.

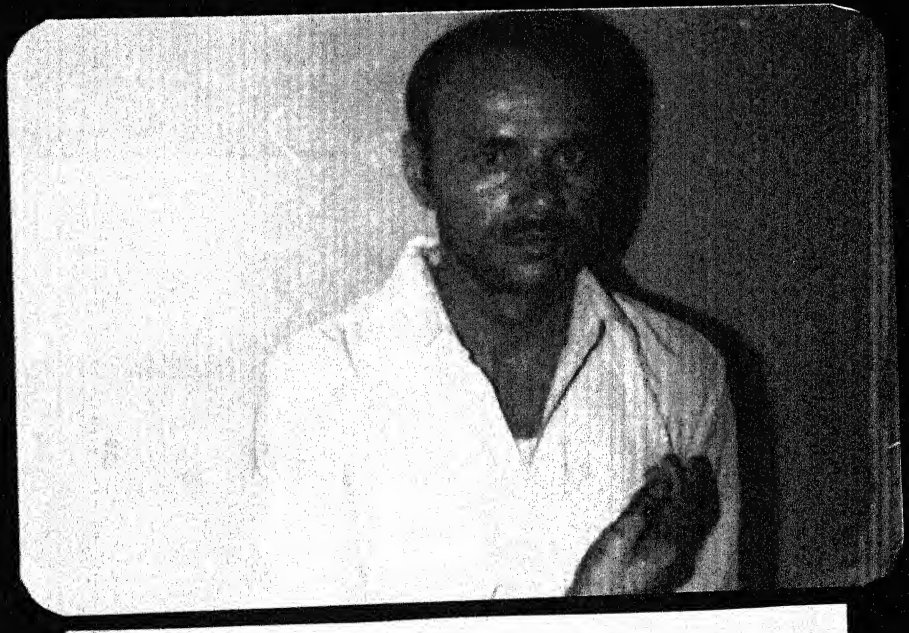
2 amniotic membrane treated cases developed contracture both involving the area of neck. One amniotic membrane treated wound and one full thickness membrane treated wound developed Keloid after complete healing.

29 Out of 36 wound surfaces were cured with good healing over the surface. Healed wound surface was pink, smooth and had flat margins. Four healed wounds had pink and raised surface with flat margins. One amniotic membrane treated wound and one full thickness treated wound had red, raised surface with flat margins. Both latter wounds developed Keloid later on.

Photograph-4: Burn wounds just before application of membrane.

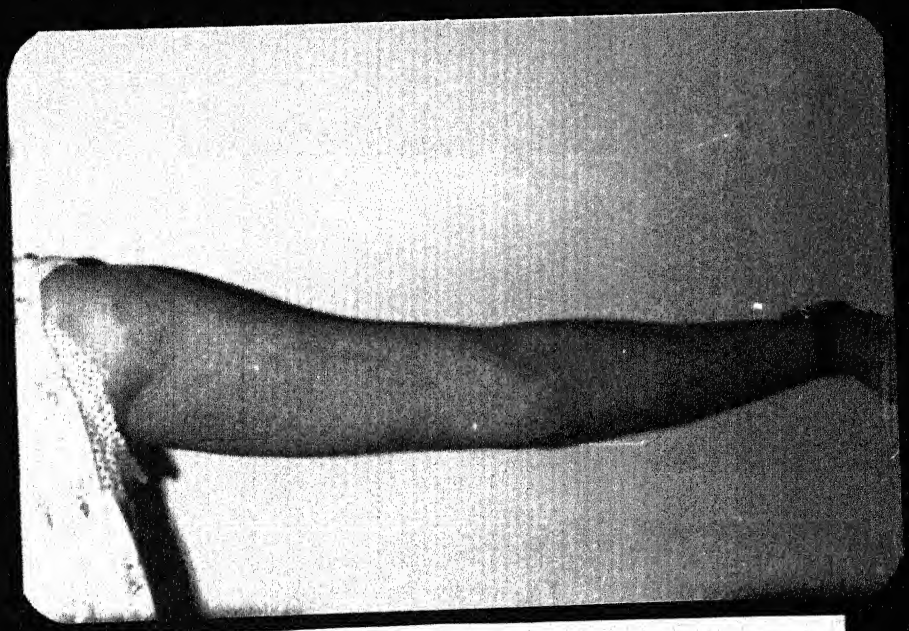
Photograph-5: Burn wounds just after application of full thickness membrane.

Photograph-6: Full thickness membrane after 36 hours of membrane application. Showing the appearance of dried membrane.



Photograph-7: Same patient as in photo-graph-4, showing complete healing of wound (10th day after application of membrane).

Photograph-8: Showing burn wounds after 24 hours of amnion application.

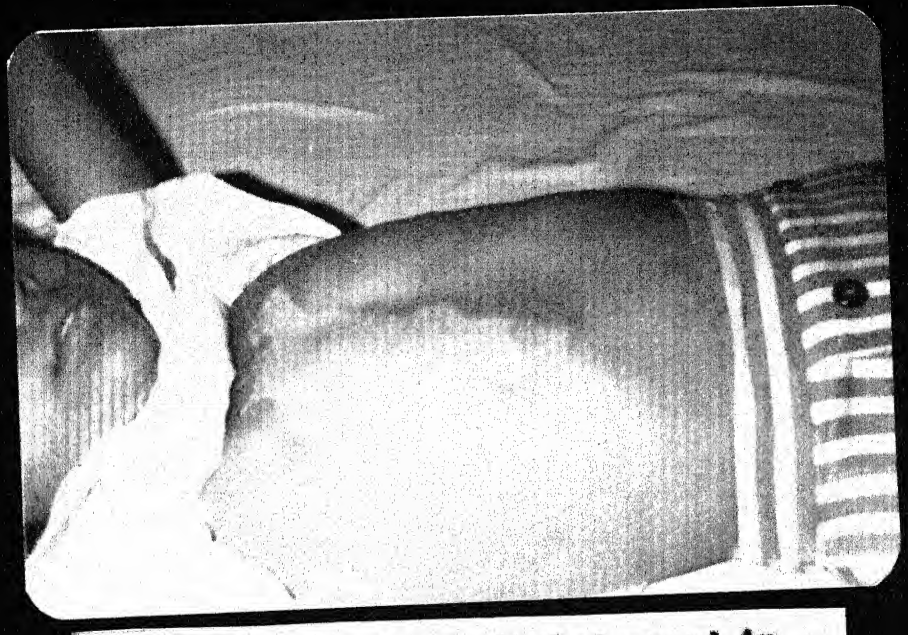


Photograph-9: Showing healed burn wound on 10th day of membrane application in the patient as shown in photograph-8

Photograph-10: Showing healed burn wound on 10th day of membrane applicati on in the patient as shown in photograph-8.

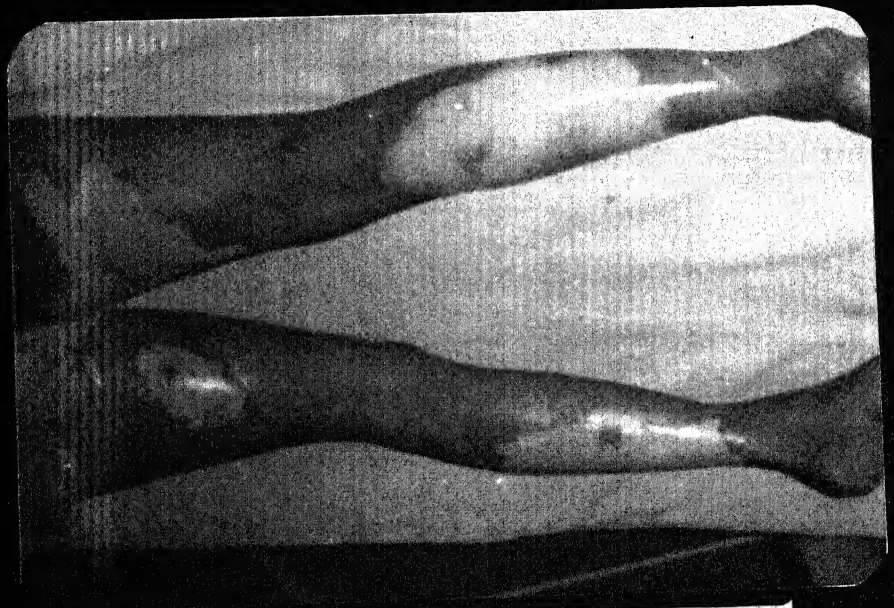
Photograph-11: Showing burn wounds immediately after application of full thickness membrane.

Photograph-12: Shows drying up of membrane (photograph taken after 36 hours of membrane application).



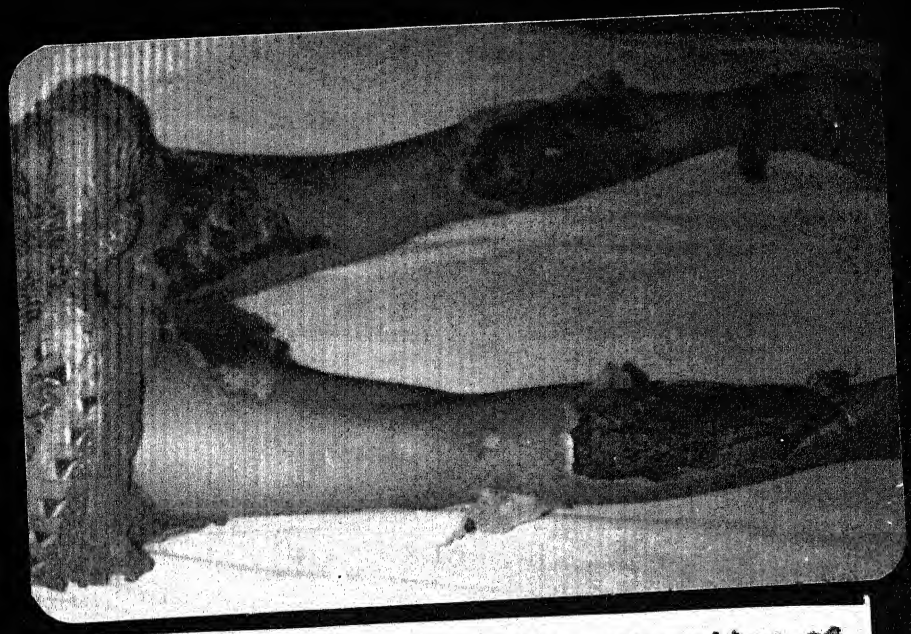
Photograph-13: Shows healed wound in the same patie-nt as in photograph-11 (photograph taken on 12th day).

Photograph-14: Shows healed wound in the same patient as in photograph -11 (photograph taken after 1 month).

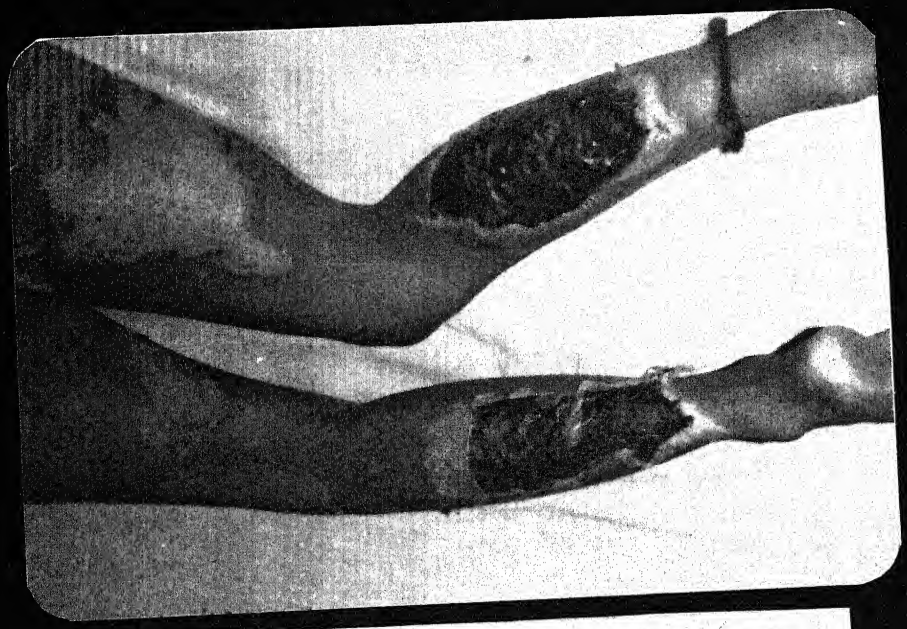


Photograph-15: Showing burn wounds immediately after amniotic membrane application.

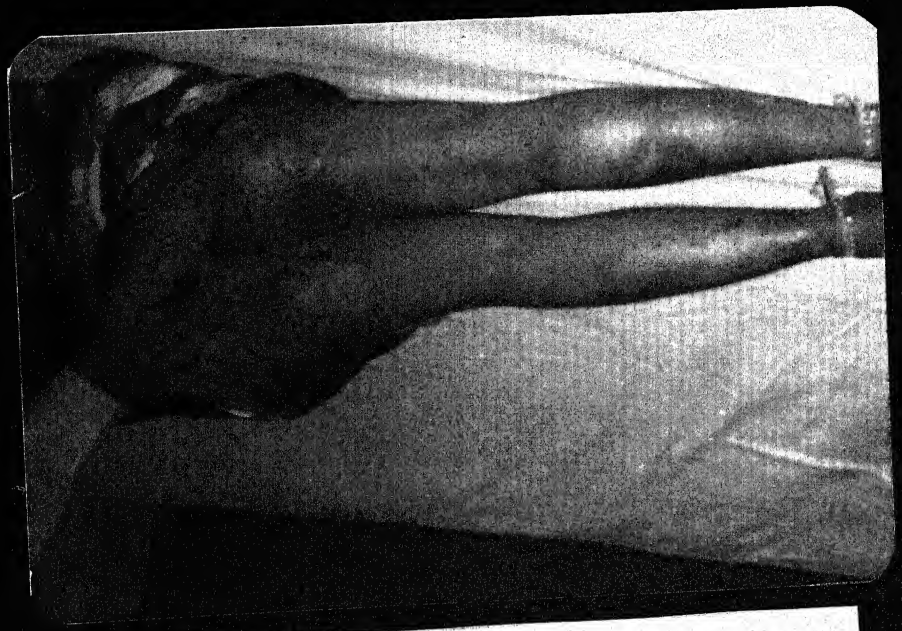
Photograph-16: Showing dried membrane on the same patient as in photograph -15. Margins of membrane shows curling up of the membrane as it is drying (photograph taken after 48 hours).



Photograph-17: Shows the seperation of membrane from the margin of the wounds (photograph taken on 11th day).



Photograph-18: Shows complete separation of membrane from the wound over thigh. Membrane applied over leg wounds in process of separation. (photograph taken on 15th day).



Photograph-19: showing healed wounds with pigmentations in same patient as in photograph-15 (photograph taken on 18th day).

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DISCUSSION

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DISCUSSION

Since the introduction of the "Biological Dressings" by Brown (1953) in the treatment of burn wounds, a number of investigators (Song 1966 , Shuck 1969, Rappaport 1970) have emphasized the effectiveness of homografts and xenografts in controlling infection and minimizing the loss of protein and fluids from the burnt surface. Silverstein (1971) and his colleages have reported xenografts to be inferior to allografts in decreasing bacterial count. They have related this failure to poorer adherence to the wound with xenografts. Rappaport and his co-workers(1970) have reported that xenografts left in place for more than 24 hours will not control the growth of bacteria. Collagen sheets are expensive and not available at every centre. A number of investigators have demonstrated the clinical feasibility of treating burn wounds with foetal membranes. Foetal membranes are easily available and are of no cost to the patient. They adhere well and obtain a biologically closed wound. Pigeon(1960) have stated that since the amniotic membrane is formed from the ectoderm of the foetus, it is like an extension of the skin of the body.

The present work is the study of the effectiveness of amnion and full thickness foetal membrane(amnion and chorion). Their results as regard the relief of pain, control of oozing and infection and rate of healing are also compared. This study was done on the patients of burns involving less than 50% of body surface area, without considering their age, sex , occupation,socio-economic status, mode and cause of injury and contamnation of wound.

As regard the incidence there was not much difference in either sex. Out of 31 cases studied, male sufferers were 13 and female were 18. 30 cases were below 30 years of age. This signifies that incidence is much higher in younger age group. This may be possible that younger persons are more engaged in daily activity, therefore they are more prone to sustain burn injury. Thermal injury appeared to be the main cause of burn. 21 cases sustained burns while cooking food, 3 cases by lamp while studying in lantern light, and 5 cases had burns due to scalding.

Total 31 cases were studied. 18 cases were treated with amnion alone, 8 cases with full thicknes membrane, and in 5 cases both amnion and full thickness membrane were applied. Thus total 36 wounds were considered.

Dino (1966) has reported the testing of many agents to sterilize the membranes and found the Normal saline with 10 lac unit of crystalline penicillin and 1 gm of streptomycin sulphate to be the best. In the present study membranes were collected from labour room and obstetrical operation theatre, and were used either fresh or were preserved in normal saline treated with 10 lac units of crystalline penicillin and 1 gm of streptomycin sulphate, at 4° c as suggested by Dino. They were used at different intervals after procurement and no difference was found in either membranes regarding their effectiveness. They retained all their biologic properties as does a fresh membrane. This suggests that both the foetal membranes can be easily collected and stored for use without changing their biologic properties. Robsen et al(1973) suggested that any subsrance chosen to sterilize the membrane should not in itself be so chemically powerful as to change biologic effectiveness of the membrane.

The use of chorion or intact amnion and chorion was suggested for deep burns as (i) it eliminates the need to separate the membranes at the time of preparation (ii)thicker intact membrane appears not to dessicate on the wound as quickly as does the amnion alone, Idem(1973) reported that bacterial growth is decreased most effectively when the biologic membrane achieve an initial 'take' onto a granulating surface. The drawback with xenograft is related to its mobility and lack in this initial 'take up'.

Amnion, chorion and combined amniotic membrane have been used by various investigators as a substitute for skin in the past. Most investigators have had a preference for one or the other of the membranes. Jenner studied amnion and chorion side by side in the same wound and found no demonstrable difference the two. Similarly Dino(1965) experimented with various layers of foetal membranes and found the end results practically the same. In the present study 23 wounds were treated with amnion alone and 13 wounds with intact Amnion and Chorion, and the results were consistent with above studies.

It was remarkable to note that pain and discomfort disappeared immediately after application of membrane. Only one out of 23 amnion treated wounds and one out of 13 full thickness membrane treated wounds required analgesics and sedatives for relief of pain proving thereby that both the membranes are helpful in decreasing pain and discomfort of the patient which is the most common symptom in a burn patient. This relief in pain is probably due to coverage of exposed nerve endings which are irritated when left exposed. Dino(1965) stated that the disappearance of pain is due to the soothing effect of the soft mucoid surface of the membrane, protecting the nerve endings

from the irritant factors which may be the only surrounding air. He further commented that since the relief is immediate it is not a chemical process like local anaesthesia which takes some time to take effect. In other words, the process could be one of mechanical barrier as afforded by the epidermis.

No allergic reaction was noted in any case, proving thereby that foetal membranes do not cause any allergic reaction when used as dressings. This is in accordance with other reports published from time to time.

Another observation was drying up of covered areas within 24-48 hours, thereby stopping oozing. The membranes adhered well to the wounds and obtained a biologically closed wound. Adherence has been proposed to be the most important property of biological membranes. According to Dino(1965) cessation of oozing is probably not due to mechanical occlusive pressure, but it is an augmentation of the co-agulum of plasma on the raw surface thereby sealing the pores. The coagulating fibrin then invades the meshes of the membrane and prevents the passage of fluid through it, at the same time making it adhere to the raw surface. The technique of applying the membrane over normal skin beyond the borders of the burn seals the periphery of the raw area.

Out of 36 wounds considered, 6 amniotic membrane treated and one full thickness membrane treated wounds developed infection. Out of these 7 wounds, 1 wound developed localized infection which was treated by splitting the membrane at that site and systemic antibiotics. In remaining 6 wounds membranes were rejected due to generalized pus collection and in all membranes were reapplied. In 2 amniotic membrane treated wounds

membranes were again rejected, probably due to failure to control infection. Culture of these infected wounds showed that staphylococcus and pseudo monas were the main organism. This difference in infection rate in 2 types of membrane treated wound is not significant because it is quite possible that (i) these wounds were not thoroughly cleaned so as to destroy the pathogens already present over the wound (ii) sterility of the membranes is not guaranteed as no culture of membranes was done after their preservation.

In the remaining 29 wounds there was no infection. This suggests that both membranes help to obtain biologically closed wound and prevent the assess of bacteria from outside, thus preventing the infection. Several authors have suggested that foetal membranes have unique antibacterial action. Allantoin, a bactericidal product of purine metabolism, immunoglobulins, and lysozymes, a bacteriolytic protein are all present in amniotic membranes and have been proposed as antimicrobial factors (Rosen 1973). Morris et al (1966) credited observed decrease in bacterial count to intimate biological closure of the open wounds by the membranes. Dino (1965) explained the absence of infection at the grafted sites as a contribution of several factors (i) the cleaning of the burn areas pregrafting must have killed or removed whatever bacteria may be there(ii) the antibiotic preservative may have killed the bacteria (iii) the sealing effect of the dried adherent membrane may have prevented the proliferation of surviving aerobic pathogens by shutting off the atmospheric oxygen (iv) the dried membrane becomes a mechanical barrier preventing the assess of bacteria in the environment into the raw burnt surface.

21 amniotic membrane treated wounds ~~within~~ healed within 25 days(3 wounds healed in 6-10 days, 10 wounds in 11-15 days, 4 wounds in 16-20 days and 3 wounds in 21-25 days). Similarly 12 full thickness membrane treated wounds healed within 25 days (7 wounds in 6-10 days, 1 wound in 11-15 days, 2 wounds in 16-20 days and 2 wounds in 21-25 days. One amniotic membrane treated wound healed in 26-30 days and one amniotic and one full thickness membrane treated wounds healed in 36-40 days. These latter 3 wounds were among those which developed infection, which could have delayed the healing process. These results show that both the membranes are equally good in promoting healing process. Early healing with membranes may be explained as a contribution of several factors (i) by covering the wounds, conversion of superficial burns to deep burns is prevented (ii) normal dermal cells which are left over the wound after injury, are prevented to destroy by covering them with membranes (iii) absence of infection is also an important factor in early and better healing . Dino (1965) observed that the crust formed under the membrane remained dry and free from infection untill their peeling off from 9th to 20th days. They became corrugated, hard and tough thus affording a good protective covering for the underlying delicate healing skin.

The healed skin treated by two membranes showed no differences. 29 healed wounds were pink,smooth and had flat margins.Four healed wounds had pink and raised surface with flat margins and two wounds had red raised surface with flat margins.Both latter wounds developed Keloid. Two cases developed contracture both involving neck area and the cause of contracture was lack of patient's co-operation.

Hansen (1950) in his study noted that enclosing a wound in plaster of paris leads to thick and raised granulation

tissue over skin margin, but with amnion grafting, granulation tissue never raises above the skin margin. Pigeon (1960) reported that (i) the normal discolouration in most cases of primary healing of burns was absent when wounds were examined after several weeks in amniotic membrane treated cases (ii) immediate protection of injured cells of dermis prevents the destruction of underlying cells, which if occur would be replace by fibrotic tissue leading to scar formation, Chao et al (1940) and Troensagaard- Hansen (1960) also have noted that amniotic membrane seemed to possess some specific healing power. They have reported a stimulation of both fibrous tissue growth and more rapid epithelial repair.

Thus it can be concluded that both foetal membranes fulfilled all the functions of an ideal biological dressings. In terms of their large size and easy availability without cost to the patient, they are actually superior to the homograft and heterograft skin. They minimized protein and fluid losses and resulted in marked relief of pain and discomfort. They appeared to increase the rapidity of epithelization. The nontoxic and non antigenic nature of the membranes as well as their adherent qualities, make it an excellent biological covering for the burn surface.

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CONCLUSION

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CONCLUSION

The effect of amniotic membrane and full thickness membrane (amnion and chorion) were studied and compared in 31 cases of burns, involving less than 50% of body surface area and following conclusions were drawn :

1. Females are 1½ times more sufferer than males for they are exposed to danger due to house work.
2. The incidence of burn is much higher in younger age group i.e. below 30 years of age.
3. Most of the burns are thermal in nature.
4. Both the membranes provide good coverage to raw area.
5. Amnion and full thickness membranes are easily collected and preserved and they can be used safely several days after preservation without changing their biological nature.
6. Both the membranes convert an open wound into biologically closed wound thus preventing protein and fluid losses from the raw surface, at the same time they prevent infection from outside.
7. They help to prevent conversion of superficial burns to deep burns thus promoting healing.
8. The discomfort and sufferings of the patients is immediately removed after membrane application which is the only distressing sympton on admission.
9. The quality of healed wounds is equally good in either membrane application. Healed wounds are pink, smooth with flat margins.

On comparing the two membranes following conclusions were drawn :

1. Full thickness membrane takes longer time to become dry in comparision to when amniotic membrane alone is applied.
2. Full thickness membrane is easy to prepare as it avoids need to separate amnion from chorion.
3. Amniotic membrane alone is superior to full thickness membrane as (i) it is fairly strong and stretchable and can cover a wider area (ii) it is not contaminated with maternal blood, therefore it can easily be cleaned.
4. Both the membranes are equally effective in alleying pain and discomfort, stopping oozing from raw surface, preventing assess of micro organism from outside into the raw surface, and promoting healing.

BIBLIOGRAPHY

1. Adams,F(1939):quoted by Hauben,D.J., Yanai,E.,Mahler,D: On the history of the treatment of burns. Burn,7:383,1981.

2. Aegineta,P(1538)quoted by Hauben,D.J.,Yanai,E.,Mahler,D: On the history of the treatment of burns.Burn,7:383,1981.

3. Agarwal,V.K.:Uses of amniotic membrane as biological dressing in superficial burn. A thesis for M.S.Surgery,Bundelkhand University, Jhansi,1982.

4. Artz,C.P.: Management of thermal burns.Mod.Med,26:181,1960.

5. Bapat,C.V.,Kothari,P:Preliminary report on acceleration of wound healing by amnion grafts. Ind.J Med.Res,62:9,1974.

6. Baxter,C.R.: Homografts and heterografts as a biologic dressing in the treatment of thermal injury. Presented at the first annual congress of the Society of Germal Plastic surgeons in Munich,Germany,September 28,1970.

7. Sushruta Samhita:quoted by Hauben,D.J.,Yanai,E.,Mahler,D: On the history of the treatment of burns.Burn,7:383,1981.

8-a. Blalock,A: Experimental shock:Possible causes for reduction in blood pressure,following mild trauma to extremity. Arch Surg., 22:598,1931.

8-b. Blalock,A: Experimental shock: Composition of fluid that escapes from blood stream after mild trauma to extremity, after trauma to intestines and after burns. Arch.Surg,22:617,1931.

8-c. Blalock,A: Experimental shock:the importance of local loss of fluid in the production of low blood presure after burns.Arch. Surg, 22:610,1931.

9. Bose,B.: Burn wound dressing with human amniotic membrane.Ann. R.Coll.Surg.Engl.,61(6):444,1979.

10. Boyer,A.(1814):quoted by Hauben,D.J.,Yanai,E.Mahler,D: On the history of the treatment of burns. Burn,7:383,1981.

11. Brown,W.H.(1896):quoted by Hauben,D.J.,Wanai,E.Mahler,D:On the history of the treatment of burns.Burn,7:383,1981.

12. Brown,J.B. and Mc,Dowell:Massive reports of burns with thick split thickness grafts.Ann.Surg,115:658,1942.

13. Brown,J.B. and Fryer,M.P.:Post mortem homografts to reduce mortality in extensive burns.J.A.M.A.,165:1163,1954.

14. Burger,K:Experimental and clinical studies on transplantation of foetal membranes.Orv Hetil,82:800,1937.

15. Burleson,R and Eisman,B:Nature of the bond between partial thickness skin and wound granulations.Surgery,72:315,1972.

16a.Burleson,R and Eisman,B: Mechanism of antibacterial effect of biological dressings. Ann.Surg,177:181,1973.

16b.Burleson,R and Eisman,B:Effect of skin dressings and topical antibiotics on healing of partial thickness skin wounds in rats.Surg.Gynoec.Obstet,136:958,1973.

17. Chao,Y.C., Humphrey,S.S. and Penfield,W.:A new method of preventing adhesions: The use of amnioplastin after craniotomy.Brit. Med.J.,1:517,1940.

18. Clowes,W(1591): quoted by Hauben,D.J.,Yanai,E and Mahler,D: On the history of the treatment of burns.Burn,7:383,1981.

19. Colocho,G,Grahm,W.P., and Green,A.E.:Human amniotic membrane as a physiological wound dressing.Arch.Surg,109:370,1974.

20. Copeland,W.P.(1887):quoted by Hauben,D.J.,Yanai,E,Mahler,D:On the history of treatment of burns.Burn,7:383,1981.

21. Dago,G.:Survival and utilization of cadaver skin.Plast Reconst. Surg,10:10,1952.

22. Davis,J.S.:Skin transplantation with a review of 550 cases at the John Hopkins Hospital.J.H.H.report,15:307,1910.

23. Davis,J.S.:Plastic surgery:Its principles and Practice.P. Blakistons son & co.Philadelphia.P.1919,1919.

24. Dahinterova,J.and Dobrokovsky,M:Treatment of the burned surface by amnion and chorion grafts.Shon.Ved.Prac.Lek.Fak.Karlov.Univ. Suppt.,51:513,1968.

25. Davidson,E.C.:Tannic acid in the treatment of burns Surg.Gynoec Obstet.,41:202,1925.

26. Davidson,E.C.:Sodium chloride metabolism in cutaneous burns and its possible significance for a rational therapy. Arch.Surg., 13:262,1926.

27. De Roth, A. :Plastic repair of conjuctival defect with foetal membranes. Arch Opth., 23:522, 1940.

28. Dino, B. R., Bufemio, G., Devilla, M et al:The use of foetal membrane Homografts in the local management of burns. J. Phil. Med. Assoc., 41:890, 1965.

29. Dino, B. R., Bufemio, G., Devilla, M et al:Human amnion: The establishment of an amnion bank and its practical applications for surgery. J. Phil. Med. Assoc., 42:357, 1966.

30. Douglas, B. :Homografts of foetal membranes as a covering for large wounds-especially those from burns; An experimental and clinical study. J. Tenn. Med. Assoc, 45:230, 1952.

31. Douglas, B., Convey, H., Stark R. B., et al:The fate of homologous and heterologous chorionic transplants as observed by the transparent tissue Chamber techique in mouse. Plast. Recons. Surg, 13:125, 1954.

32a. Dupuytren. G. :Lecons orales dee Clinique chirurgicale, Paris Bailliore. Vol 1 p 413, & Vol. 2 p-1, 1832.

32b. Dupuytren, G. :Clinical lectures in surgery. Delivered at Hotel Dieu in 1832(Sidney Doane A transt.)Bostan centre, Hender & co., p. 236, 1832.

33. Dzondi, K. H. (1816) :quoted by Hauben, D. J., Yanai, E., Mahler, D:On the history of treatment of burns. Burn, 7:383, 1981.

34. Eade, G. G. :The relationship between granulation tissue, bacteria and skin grafts in burned patients. Plast Recons. Surg., 22:42, 1958.

35. Earle, J. (1799) :quoted by Hauben, D. J., Yanai, E. Mahler, D. : On the history of the treatment of burns. Burn, 7:383, 1981.

36. Ebers, G. (1875) :quoted by Hauben, D. J., Yanai, E., Mahler, D: On the history of the treatment of burns. Burn, 7:383, 1981.

37. Elhans, I. P. et al:Role of collagen sheet in the management of burn wound. A thesis for M. S. examination, Agra University, Agra, 1978

38. Fabricius, W. H. (1967) :quoted by Hauben, D. J., Yanai, E., Mahler, D. :On the history of the treatment of burns. Burn. 7:383, 1981.

39. Fox, C. L. Jr, Rappole, B. W., Stenford, W. T. :Control of pseudomonas infection by silver sulphadiazine. Surg. Gynaev. Obstet., 128:1021, 1969.

40. Fox,C.L.Jr,Rappole,B.W.,Stenford,W.T.:Silver sulphadiazine for control of burn wound infection.International Surgery,60:5,1975.

41. Galask,R.P.,Snyder,I.S.:Antimicrobial factors in amniotic fluid. Ann.J.Obstet.Gynaec,106:59,1970.

42. Girdner,J.H.:Skin grafting with graft taken from the dead subjects. Med.Rec.N.Y.,119:1881.

43. Gupta,A.K.,Chaturvedi,S.N.,Samuel,K.C. et al:Preliminary reports of use of collagen sheets as a primary dressing over wound with skin loss. Paper presented at the annual conference(Plastic Surgery Section)held at Trivendrum Feb/March,1974.

44. Hansen,E.,Troensegaard:Amniotic grafts in chronic skin ulceration.Lancet,1:859,1950.

45. Hansen.E.,Troensegaard(1956):quoted by Agrawal V.K.,1982.

46. Hanson,E.,Troensegaard:Peripheral vascular disesses treated by amnion implant:A Review of 100 cases.Lancet,1:520,1960.

47. Hebra,F.V.:Hebra on diseases of the skin,London.(Fagga,C.H. transl,1860,Vol-I.pp.316)

48. Hebra,F.V.(1861)quoted by Hauben,D.J.,Yanai,E.,Mahler,D.:On the history of the treatment of burns.Burn,3:383,1981.

49. Heister,L(1743):quoted by Hauben,D.J.,Yanai,E.,Mahler,D.:On the history of the treatment of burns.Burn,3:383,1981.

50. Idem:Predicting skin graft survival.J.trauma,9:374,1971.

51. Ivanova,S.S.:The transplantation of skin from dead body to granulating surface.Ann,Surg.,12:354,1890.

52. Jain,R.K. et al: An enquiry into role of collagen based surgical in the wounds and burns healing.Thesis for M.S.examination, Meerut University,Meerut,1976.

53. James,J.H., and Eatson,A.C.:The use of opsite, a vapour permeable dressing on skin graft donor sites.Brit.J.Plast Surg,28:107,1975.

54. James,A.O.'Neel,Jr.et al:The extended use of skin homografts Arch.Surg.,99:263,1969.

55. Junzekovic,Z.:Present clinical aspects of burns.Mariber,99,215, 1868.

56. Jenner,J.A.:The grafting of preserved foetal membrane to denuded skin surface.Marquette University Milwaukee,Wisconsin.

57. John.D.,Trelford, and Marilyn.Trelford-Souder:The amnion in surgery past and present.Ann.J.Obstet.Gynaec,134(7):833,1978.

58. Jullian,A.Sterling:Use of amniotic membranes to cover surface defects due to flame burns.Ann.J.Surg.,91:940,1956.

59. Katnstan(1941):quoted by Agarwal,V.K., 1982.

60. Kantish,E.: An Essay on burns,Londan,1817,pa.102.

61. Kornberg,J.,Buns.H.E.,Kofesjiam,R.et al:Ultra thin silicone polymer membrane:A new synthetic skin substitute.Trans.Amm.Soc. Artif.Inter.Organ,18:39,1972.

62. Kothari,P:Total glossectomy and repair with amniotic membrane (Preliminary observations).India Med.Assoc.J.,62:87,1974.

63. Kubani,A.:Trapianto et'amnnon sterile ottennto dal teglio cesario. Ann.Ital.Clir,25:10.1948.

64. Lanke,L.O.,Milson,G.E. and Reithner,H.L.:The evaporative water loss from burns and the water vapour permeability of grafts and artificial membranes used in the treatment of burns.Burn,3:159, 1977.

65. Liedberg,N.C.F.,Reiss,E. and Artz,C.P.:Infection in burns 111. Septicaemia, a common cause of death.Surg.Gynec,Obst,99:151,1954.

66. Lusgarten(189):quoted by Hauben,D.J.,Yanai,E. Mahler,D.: On the history of the treatment of burns.Burn,7:383,1981.

67. Martin,C.,Roben,M.C.:The effect of human amniotic membrane on the bacterial population of infected rat burns.Ann.Surg.,173:144, 1973.

68. Martin,C.Roben,M.C. et al:Clinical experience with amniotic membrane as a temporary biological dressing.Year book of Plast. Recars.Surg,1976.

69. Massee,J.S.,Symmonds,R.E.,Dockorty,M.B. et al:Use of foetal membrane as replacement for pelvic periotonium after pelvic exanteration in the dog.Gynae Obstel,23:407,1962.

70. Miller,J:Early homografts of second degree burn wounds.Clin Plast.Surg,1:563,1974.

71. Monerief,J.A.:Topical antibacterial therapy for burn wounds.Clin. Plast Surg,1:563,1974.

72. Monerief,J.A. et al.:The management of burns I general consideration and sulfamylon method II The silver nitrate,method.

73. Morris,P.J.,Bondoc,C. and Burke,J.E.: The use of frequently changed skin allografts to promote healing in the non healing infected ulcers.Surgery 60:13,1966.

74. Moyer,C.A.,Brontano,L.,Gravenes,D.L. et al: Treatment of large human burns with 5% silver nitrate solution.Arch.Surg.90:812, 1965.

75. Oppenheimer,L.S.:The treatment of burns,N.K.Med.J.,84:646,1906.

76. Pickrell,K.L.:A sulfonamide film for use as a surgical dressings. Bull John Hopkins Hosp.,71:304,1942.

77. Pigeon,J.:Treatment of second degree burns with amniotic membranes.Can.Med.Assoc.J,83:844,1960.

78. Pinkerton,M.C.:Amniotlastin for adherent digital flexer tendons.Lancet,1:70,1942.

79. Pollock:Quoted by Fresh water,M.F. and Krizek,T.J.: Skin grafting of burns.A centennial.J.Trauma,11:862,1971.

80. Pringle,R:Amniotic implantation in peripheral vascular disease. Lancet,1:77,1963.

81. Rappaport,I,Pepino.A.T.,Dietrick,W:Early use of xenografts as a biologic dressings in burn trauma.Am.J.Surg,120:144,1970.

82. Reid,A.: The treatment of burns and other surface wounds. Lancet,1:677,1898.

83. Rhazes(1556)quoted by Hauben,D.J.,Yanai,E.,Mahler,D: On the history of the treatment of burns.Burn,7:383,1981.

84. Robsen,M.C.,Krizek,T.J.,Koss,N et al:Amniotic membrane as a temporary wound dressing.Surg.Gynaec.Obstet,136:904,1973.

85. Rowling(1958):Quoted by Hansen.Traneugaard,1960.

86. Sabella,N.:Use of foetal membranes in skin grafting:Med.Rec., N.Y.,83:478,1913.

87. Salisbury,R.E.,Wilmore,D.W.,Silverstein,P. et al:Biological dressing for skin grafting donor sited.Arch Surg.,106:705,1973.

88. Sharma,et al:Evaluation of use of preserved skin homografts in patient of burns.Thesis for M.S.examination surgery.Agra University,Agra,1978.

89. Shanker,M.L.:A clinical study on the use of collagen sheet in the management of burn wounds.Thesis for M.S.examination. Surgery.Banglore University,Banglore,1975.

90. Shede,M.,VII Vereins chronik:Arztlicher verein zu Hamburg. Sitzung Von 25,Januer 1881,Dtrch Med Wochenchr,7:352,1881.

91. Shuck,J.M.,Pruitt,B.A.,Moncreif,J.A.:Homografts skin for wound coverage.A study in versatility.Arch.Surg.,78:472,1969.

92. Silverstain,P.,Curreri,P.W., and Munster,A.M.:Evaluation of fresh viable porcine,cutaneous xenografts as a temporary burn wound cover. Annual Progress Report,U.S.Army Institute of surgical Research,1970-71.

93. Sinha,R.M., Verma,P.K. and Madam,P.:Collagen sheet as a biological dressings in burns.Ind.J.Plast Surg.5(2):1972.

94. Sneve,H.: The treatment of burns and skin grafting.J.Am.Med. Assoc.,45:1,1905.

95. Song, I.C., Bromberg,B.E.,Mohni,M.P. et al:Heterografts as a biological dressings for large,skin wounds.Surgery,59:576,1966.

96. Tavis,M.J.,Thornton,J.W.,Harmvey,J.H. et al: Graft adherence to the de-epithelized surfaces:A comparative study.Ann.Sur.,184: 594,1876.

97. Thornton,J.W.,Tavis,M.J.,Harmvey,J.H. et al:Grafts adherence to wound surface:Collagen fibrin interactions.Burns,3:23,1977.

98. Tommasoli(1897): Quoted by Agarwal,V.K.,1982.

99. Townsend,P.L.G.: The quest for a cheap and painless donor site dressings. Burns, 2:82,1977.

100.Trelford.J.D.,Anderson,P.G.,Hansen,F.W. et al:Amnion autograft a.and allograft as a cover for skin defects in sheep,J.Med,31:81, 1972.

101b.Trelford,J.D,Anderson,D.G.,Hansen,F.W. et al:Considerations of the amnion as an autografts and allografts in sheep.J.Med,31: 231,1972.

102.a Trelford,J.D.,Hansen,F.W. and Anderson,D.G.:Amniotic membrane as living surgical dressing in human patients. Onchology,28:358,1973.

102 b. Trelford,J.D.,Anderson,D.G.,Hansen,F.W.:Amniotic membrane used for radical vulvectomies,Gynae.Obsetet,12:1,1973.

102c.Trelford,J.D.,Hansen,E.W., Anderson,D.G. et al:Implanted amniotic membrane as an autegraft and as an allograft,J.Med. 6:169,1975.

103b. Trelford,J.D.,Hansen,F.W.,Anderson,D.G. et al:Amnion autograft, permanent structure,J.Med,6:243,1975.

103.c.Trelford?J.D.,Hansen,F.W. amd Anderson,D.G.:Wound healing and the amniotic membrane,J.Med.6:383,1975.

104. Trelford-Souder.M., and Trelford,J.D.:Replacement of the peritonium with amnion following pelvic exenteration.Surg. Gyneec. Obstet.,145:699,1977.

105. Trelford Souder.M. et al: Use of allograft amniotic membrane for control of intra abdominal adhesions.J.Med,9:273,1978.

106. Valesco de Torenta(1490):Quoted by Hauben, D.J.,Yanai,E., Mahler,D. : On the history of the treatment of burns.Burn, 7:383,1981.

107. Underhill,F.P.: The significance of anhydraemia in extensive superficial burns.J.Am Med Assoc.,91:852,1930.

108. Unger-Hamilton(1958):Quoted by Hansen-Transeugaard,1960.

109. Wallace,A.B.:Treatment of burns:A return to basic principles. Br,J.Plast Surg.,2:232,1949.

110. William,W.Monofo, Som,N.,:Tandon:Cerium nitrate:A new topical antiseptic for extensive burns.Surg.,80(8)1975.

111. Wilms.M(1901): Quoted by Houben?D.J.,Yanai,E.,Mahler,D: On the history of the treatment of burns.Burns,7:383,1981.

112. Wiseman,R.C.(1676): Quoted by Houben,D.J.,Yanai,E.Mahler,D. On the history of the treatment of burns.Burn,7.383,1981.